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Identification of *Phytophthora* spp. from Perennial Crops in Malaysia, Its Pathogenicity and Cross-pathogenicity

(Pengecaman Pencilan *Phytophthora* spp. daripada Tanaman Saka Berkayu di Malaysia, Kepatogenan dan Silang Kepatogenannya)

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ABSTRACT

Phytophthora *is one of the most important plant pathogens of perennial crops in the tropics. This study characterised the pathogen(s) responsible for cocoa black pod rot, rubber pod rot and durian stem canker. Eighteen* Phytophthora *isolates were collected from several states in Malaysia, namely Pahang, Johor and Selangor. A total of 12* Phytophthora palmivora *and six* Phytophthora nicotianae *isolates were isolated and identified based on the morphological and molecular characteristics. Internal transcribed spacer (ITS) sequences enabled* Phytophthora *identification to species level. Inoculation of 18 isolates on detached leaves and unripen cocoa pods successfully demonstrated the progressive development of lesions on its original host. Lesions were also observed in all wounded detached leaves of young durian (clone D24) and rubber (clone RRIM600) and unripen cocoa pods (clone BAL244) regardless of host and isolates.* Phytophthora *isolates from durian (DSCI) and rubber (R4A) used to inoculate cocoa pods exhibited lesion sizes of 11.6±0.75 and 9.6±0.64 mm, respectively. However,* Phytophthora *isolates from cocoa (CPR25) developed a longer length of lesion of 18.6±0.47 mm. On durian leaves,* Phytophthora *isolate from rubber (R4A) and cocoa (CPR25) recorded 11.73±1.04 and 5.22±0.57 mm length of lesion while* Phytophthora *isolates from durian (DSCB4) produced 13.13±1.29 mm lesion on its native host. Isolates from cocoa (CPR22) and durian (DSCE3) infected rubber leaves with 3.74±0.48 and 2.55±0.31 mm length of lesion, in comparison to rubber isolates (R1B) with 5.43±0.23 mm length of lesion. Differences in the length of lesion demonstrated higher level of virulence on the native host.*

Keywords: Cross-pathogenicity; internal transcribed spacers (ITS); pathogenicity; phylogenetic tree; Phytophthora

ABSTRAK

Phytophthora *merupakan patogen tumbuhan yang penting kepada tanaman saka berkayu di kawasan beriklim tropika. Pencirian dan pengenalpastian terhadap patogen* Phytophthora *spp. yang merupakan penyebab penyakit buah hitam pada tanaman koko, reput pada buah getah dan kanker batang pada pokok durian telah dijalankan di dalam kajian ini. Sejumlah 18 pencilan* Phytophthora *spp. telah diperoleh daripada sampel tisu berpenyakit dari beberapa buah negeri di Malaysia iaitu Johor, Pahang dan Selangor. Dua belas pencilan telah dikenal pasti sebagai* Phytophthora palmivora *dan enam lagi pencilan sebagai* Phytophthora nicotianae *berdasarkan sifat morfologi, kultur dan pencirian secara molekul. Pencirian molekul pada kawasan jujukan* internal transcribed spacer *(ITS) membolehkan pengecaman* Phytophthora *sehingga ke peringkat spesies. Inokulasi 18 pencilan* Phytophthora *spp. pada helaian daun dan buah koko yang belum matang menunjukkan kesan lesi merebak pada sampel berkenaan. Kesan lesi hadir pada kawasan luka helaian daun muda durian (klon D24), getah (klon RRIM600) dan buah koko yang belum matang (klon BAL244) tanpa mengambil kira faktor hos dan pencilan. Pencilan* Phytophthora *daripada durian (DSCI) dan getah (R4A) yang digunakan untuk menginokulat buah koko yang belum matang telah mempamirkan saiz lesi masing-masing 11.6±0.75 dan 9.6±0.64 mm. Walau bagaimanapun, pencilan* Phytophthora *daripada koko (CPR25) mempamerkan kesan lesi yang lebih panjang (18.6±0.47 mm). Pada daun durian, pencilan* Phytophthora *daripada getah (R4A) dan koko (CPR25) telah mempamerkan kesan lesi bersaiz masing-masing 11.73±1.04 dan 5.22±0.57 mm. Sementara itu, pencilan* Phytophthora *daripada pencilan durian (DSCB4) telah menghasilkan saiz lesi 13.13±1.29 pada hos asalnya iaitu daun durian. Pencilan daripada koko (CPR22) dan durian (DSCE3) yang diinokulat pada daun getah telah mempamerkan saiz lesi masing-masing 3.74±0.48 dan 2.55±0.31 mm. Namun, lesi yang lebih panjang (5.43±0.23 mm) diperhatikan pada daun getah yang diinokulat dengan pencilan (R1B) daripada getah. Perbezaan panjang lesi yang diperhatikan telah menunjukkan tahap kevirulenan yang tinggi pada perumah asalnya.*

Kata kunci: Internal transcribed spacer (ITS); kepatogenan; Phytophthora*; pohon filogeni; silang-kepatogenan*

INTRODUCTION

Phytophthora is a 'fungus-like' plant pathogen under the kingdom Chromista. It is oomycetes and synonymous as a 'plant destroyer'. *Phytophthora* causes various devastating diseases in many different types of plants including annual and perennial crops in tropical, sub-tropical and temperate regions. It has been reported to cause a huge impact on forest ecosystems as *P. cinnamomi* causing 'Jarrah dieback' resulting widespread decline of the dominant forest species of *Eucalyptus marginata* (jarrah) (Hee et al. 2013). In Western Australia, *P. cinnamomi* is known as a biological bulldozer as 2284 of 5710 plant species are susceptible or highly susceptible (Shearer et al. 2004). In contrary, *P. fragariae* var. *rubi* only infects a single host, raspberry root (Kennedy & Duncan 1995). Commonly present in Malaysia, *P. palmivora* and *P. nicotianae* (synonym *P. parasitica*) are known to be infective to a wide range of hosts - *P. nicotianae* infects horticultural and commodity plants such as rubber, citrus, tobacco and tomato while *P. palmivora* is a serious pathogen of economically important crops such as cocoa, rubber, coconut and durian (Drenth & Sendall 2004). These suggested that *Phytophthora* species vary greatly in their degree of host specificity. It is a flexible and very effective pathogen due to its uncommon genetic architecture that enables *Phytophthora* to cause rapid evolution in pathogenicity (Jiang et al. 2008; Raffaele et al. 2010; Seidl et al. 2011).

A rapid and precise identification of *Phytophthora* spp. are important for disease management especially for quarantine purposes and during pre-planting restricting the spread of *Phytophthora* disease through plant materials (MacDonald et al. 1990). Traditionally, production of oogonia, antheridia and oospores (sexual spores) and the morphology of asexual spores (zoosporangium and chlamydospores) produced by *Phytophthora* spp. are used as the characteristics for species identification. Newhook (1978), Stamps et al. (1990) and Waterhouse (1963) agreed that although both of these asexual and sexual spores have varying and very little morphological differences among the species, they have remained as the basis for species identification and taxonomy of *Phytophthora* species.

To date, molecular identification is a more rapid, sensitive, and applicable method to identify *Phytophthora* spp. compared to morphological identification. DNA sequence analysis of the internal transcribed spacer (ITS) of ribosomal DNA have been employed for the detection, identification, classification, and phylogenetic analysis of many oomycetes at the species level (Chillali et al. 1998; Cooke et al. 2000; Henson & French 1993; Hibbett 1992; Oliver 1993; Taylor et al. 2000). The ITS is particularly useful for species discrimination because they evolve in a neutral manner at a rate that approximates the rate of speciation (Lee & Taylor 1992; White et al.1990). Lee and Taylor (1992) demonstrated that ITS sequences have been successfully used to differentiate tropical *Phytophthora* spp. such as *P. palmivora*, *P. megakarya*, *P. capsici*, *P. citrophthora* and *P. cinnamomi*.

The potential cross-infection of *Phytophthora* spp. to susceptible potential host plants has not been extensively studied in Malaysia. This is particularly important in intercropping systems, for instance, farmers planting durian trees as shade plants for cocoa trees, as both of these plants have the potential to be infected by *Phytophthora* spp. Therefore, the objectives of this study were: to isolate and characterize *Phytophthora* spp. associated with cocoa and rubber pod rot, and durian stem canker using morphological characteristics; to identify each *Phytophthora* spp. using ITS sequences to support the results of morphological identification; and to determine the pathogenicity and cross-pathogenicity of the identified *Phytophthora* spp.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PHYTOPHTHORA ISOLATION

Phytophthora isolated from rubber were collected from infected blackened green pods with malformed and unopened seeds, which were shriveled and rotten (Figure 1(A)). Cocoa isolates were from cocoa pods exhibiting symptoms of firm brown or black lesions with a well-marked boundary (Figure 1(B)). Durian stem cankers appearing as wet lesions with bark discoloration and exuding reddish-brown resinous substances with necrosis were also collected (Figure 1(C) & 1(D)). Isolates were obtained from diseased fruits and stem tissues (0.5 cm) from the advancing margins. The infected fruits and tissues were cut by sterilized scalpels. The samples were washed and surface sterilized by soaking in 10% sodium hypochlorite (NaOCl) for 30-60 s followed by rinsing (twice) with sterile distilled water and air-dried on sterilized filter papers. Four pieces of these tissues were placed on P10VP medium (Tsao & Ocana 1969). Pure culture obtained from infected tissues were maintained on corn meal agar (CMA) slants at 24°C and kept for further studies. Single-spore isolation of germinated cyst (a zoospore that has lost the flagella) and hyphal tip technique were applied in order to get pure culture (Erwin & Ribeiro 1996; Ko & Ho 1997).

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

Mycelial plugs (6 mm) from the advancing edges of 5–day–old CMA cultures of the *Phytophthora* isolates were placed at the center of carrot agar (CA), vegetable juice (V8) agar (VJA), potato dextrose agar (PDA) and CMA plates in three replicates. These plates were incubated in continuous darkness at 28±1.5°C and colony characteristics were compared after seven days. Another 6 mm mycelial plugs placed onto CMA and incubated in the dark at 16, 20, 24, 28, 32 and 36°C with six test plates for each temperature. Data was collected after five days by measuring the colony diameter and analyzed with Statistical Analysis System (SAS) software version 9.3.

Sporangium dimension and depth of apical thickening were obtained from plugs of test isolates grown on 10 %

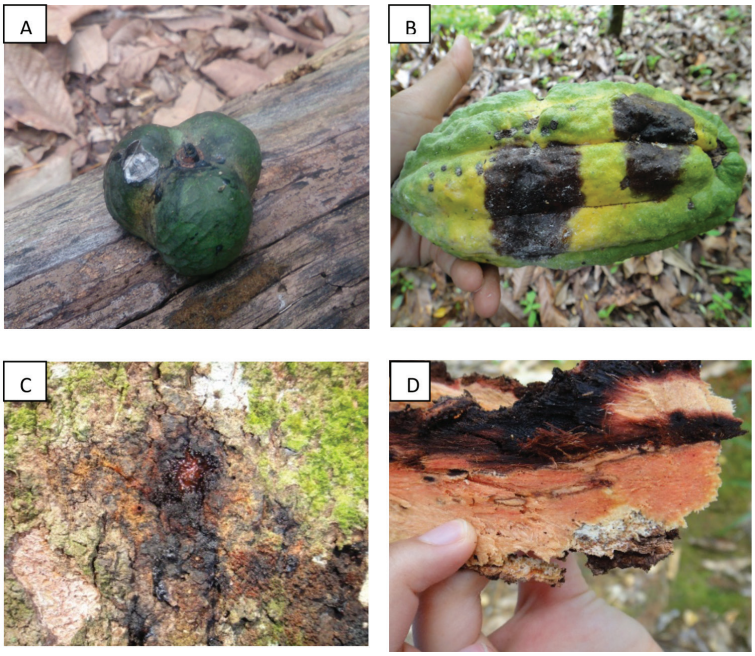

FIGURE 1. Green rubber pod infected severely by *Phytophthora* spp. becomes black, unopened, with the seeds inside shriveled and rotten (A). A symptom of *Phytophthora* pod rot is firm rot that can be distinguished from pod rot caused by *Botryodiplodia theobromae* and infected by *Colletotrichum* spp. (B). Initial symptom of canker; the appearance of wet lesions on the bark (C). The tissues and wood appear dull and discoloured from cream coloured to reddish brown when the bark is stripped away (D)

V8 agar (V8A) for five days. These plugs were flooded with a shallow layer of distilled water and placed under continuous fluorescent light and incubated at 28±1.5°C for 48 h (Erwin & Ribeiro 1996). Drops of sporangia suspension were mounted onto clean glass slides in lactophenol blue to facilitate measurements of its length, breadth and depth of apical thickening. Observations and measurements were done via microscope eye-piece camera (Dino-Eye AM4023X) with Dino Capture 2.0 software.

To determine the persistence (caducity) of sporangia, sporulating agar plugs of the mycelia was removed from the liquid culture, placed it in contact with a microscope slide containing a drop of water and agitated it briskly to dislodge the sporangia. There will be many dislodged sporangia with pedicels seen under microscope if the species has caducous sporangia features. Pedicel length was also measured using the microscope eye-piece camera (Dino-Eye AM4023X). Separate sporangial suspension for cold treatment at 6°C for 25 min was done and the suspension was vortexed for 2 min (Lee & Varghese 1974). Subsequently, zoospores were produced 20 min after the suspension was brought to room temperature. The width of the exit pore of the sporangium was measured.

Four to five mycelial plugs of the test isolate were plated in a 90 mm petri dish containing 20 mL of 10% V8 incubated under fluorescent light at 25±1.5°C. Chlamydospores were produced after 14 days of incubation. The chlamydospores were harvested by washing the culture with deionized water and vortexing at a medium speed for 5 min. The resulting suspension of chlamydospores, sporangia and hyphae was strained through two layers of muslin and centrifuged at 1800 g for 10 min. The supernatant was decanted and the concentrated suspension was vortexed again before being filtered through two sheets of tissue paper to separate the chlamydospores and sporangia from the hyphae. The method for chlamydospore production was based on Chee's method (1973) with some modification. The diameter of the chlamydospores was measured using a microscope eye-piece camera (Dino-Eye AM4023X).

EXPERIMENTAL DESIGN AND DATA ANALYSIS

Measurements of sporangium dimensions (apical thickening, pedicel length and width of exit pore of sporangia) and chlamydospore diameters were based on 100 sporangia of the test isolates in separate experiments. Recorded data were analyzed using SAS version 9.3.

DNA EXTRACTION

Three to four mycelial plugs from 6–day–old cultures were transferred into 30 mL clarified 10% V8 broth medium and incubated at 28±1.5°C for six days. The mycelium was filtered through a double layer of sterile muslin, washed twice with sterile distilled water, drained on filter paper, and ground in liquid nitrogen with a mortar and pestle. Genomic DNA was extracted from all *Phytophthora* isolates obtained from 200 mg ground mycelia via the CTAB method (Innis et al. 1990). The quality and concentration of the genomic DNA were assessed using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

PCR AMPLIFICATION

A universal primer pair nucleotide sequence was used to amplify the ITS region: ITS6 (5′–GAAGGTGAAGTCGTAACAAGG–3′) and ITS4 (5′–TCCTCCGCTTATTGATATGC–3′) (Cooke et al. 2000). Amplification reactions were prepared to a total volume of 25 µL containing 12.5 µL Dream *Taq* Green PCR Master Mix (2X) supplied with 2X Dream *Taq* Green Buffer, 0.4 mM each dATP, dCTP, dGTP and dTTP, 4 mM $MgCl_2$ (Thermo Saintific, USA), 1 µL each of forward and reverse primers (concentration of each primer was 1 µM), 40 ng template DNA and 6.5 µL nuclease-free water. PCR amplifications were performed in BioRad Thermocycler (C1000 Touch™). The amplification protocol included initial denaturation for 3 min at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 52.65°C for 30 s and extension at 72°C for 1 min and with a final extension at 72°C for 10 min. A 100-bp ladder (First Base Laboratories Sdn. Bhd., Selangor, Malaysia) was used as a marker. Amplified products were separated via electrophoresis (70 Vcm–1 for 50 min) in 1.5% (w/v) agarose with 1× TAE buffer. The gels were stained with 2 µL Florosafe DNA stain (First Base Laboratories Sdn. Bhd., Malaysia) for visualization under UV light using a gel documentation system (Bio-Rad, Philadelphia, PA, USA).

SEQUENCE ANALYSIS

To examine the sequence alignment, we compiled nucleotide sequences in the FASTA format and compared it to the deposited sequence in the GenBank via BLAST sequence alignment was performed using BioEdit software version 7.0.5 (Hall 1993). In addition, we constructed a phylogenetic dendrogram based on the Neighbor–Joining (NJ) method with MEGA software (version 6) (Tamura et al. 2013). The bootstrap values illustrated on the phylogenetic tree was generated based on a 1000 replicate heuristics search.

PATHOGENICITY AND CROSS-PATHOGENICITY TESTS

A total of 18 *Phytophthora* spp. isolates from cocoa, durian and rubber were tested for pathogenicity against its own host. These isolates were also tested for cross-pathogenicity against leaves from young durian (clone D24) and rubber (clone RRIM600) and unripe cocoa (clone BAL244) pods through artificial inoculation on wounded tissues.

All pathogenicity and cross-pathogenicity studies were carried out *in vitro* on detached unripe cocoa pods and leaves of young durian and rubber. Each isolate was tested in five replicates. The 5 mm inoculum plug was placed at the center of the leaflets and cocoa pods for artificial inoculation. Inoculum for the trials were obtained from 8–day–old *Phytophthora* spp. cultures exhibiting many sporangia. The samples were enclosed in moistened polyethene bags and incubated it individually at 24±1.5°C. Successful infection was determined at 3-5 days of incubation by measuring the extent of discoloration developing around the plugs. As a control treatment, two replicates were prepared and all tissue samples were treated with 6 mm V8 agar plugs without the fungus. The experiments were conducted in a completely randomized design (CRD). Recorded data were analyzed using SAS. The data was analyzed using Tukeys's Studentized Range (HSD) test to determine significance at a 5% probability level.

Disease Severity Disease severity (DS) of leaf and pod were expressed according to the formula given:

$$\text{DS}\,(\%) = \frac{\sum (\text{Number of samples in the scale} \times \text{Severity Scale}) \times 100}{\text{Total number of sample assessed} \times \text{Highest scale}}$$

A disease severity scale was constructed to assess disease severity exhibited by samples subjected to pathogenicity test conducted via *in-vitro* test. The scales were made as Figures 7-9.

Re-Isolation The pathogen was re-isolated from the margins of infected young durian and rubber leaves and detached cocoa pods on CMA to confirm *Phytophthora* spp. as the pathogen causing the lesions. After seven days of incubation, the pathogen was successfully isolated and any similarity was compared to the previously inoculated pathogen.

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERISTICS

Twelve isolates from cocoa and durian were identified as *P. palmivora* and six isolates from rubber as *P. nicotianae* (synonym *P. parasitica*). *Phytophthora* isolated from rubber produced fluffy colonies with irregular margins (Figure 2C), meanwhile isolates from cocoa and durian produced stellate and striate colonies with sparse aerial mycelia and well-defined margins on CA, VJA, CMA and PDA (Figure 2(A) & 2(B)). Isolates from cocoa and durian grew at a temperature range16-32°C. However, it was not able to grow at 36°C. Nevertheless, isolates from rubber were able to grow at a temperature range 16-36°C. In general, all isolates grew at an optimum temperature 28°C (Table 1). Morphological and cultural characteristics of the isolates from cocoa and durian in the present study similar to the description of *P. palmivora* by Brasier and Griffin (1979), Newhook (1978) and Waterhouse et al. (1983). These results were supported by the failure of *P. palmivora* isolates from cocoa and durian to grow at 36°C or higher, further distinguishing it from *P. nicotianae* isolated from rubber which could grow well at 35°C or higher (Newhook 1978; Waterhouse et al. 1983; Weste 1983). However, both *P. palmivora* and *P. nicotianae* were capable of producing abundant chlamydospores on agar

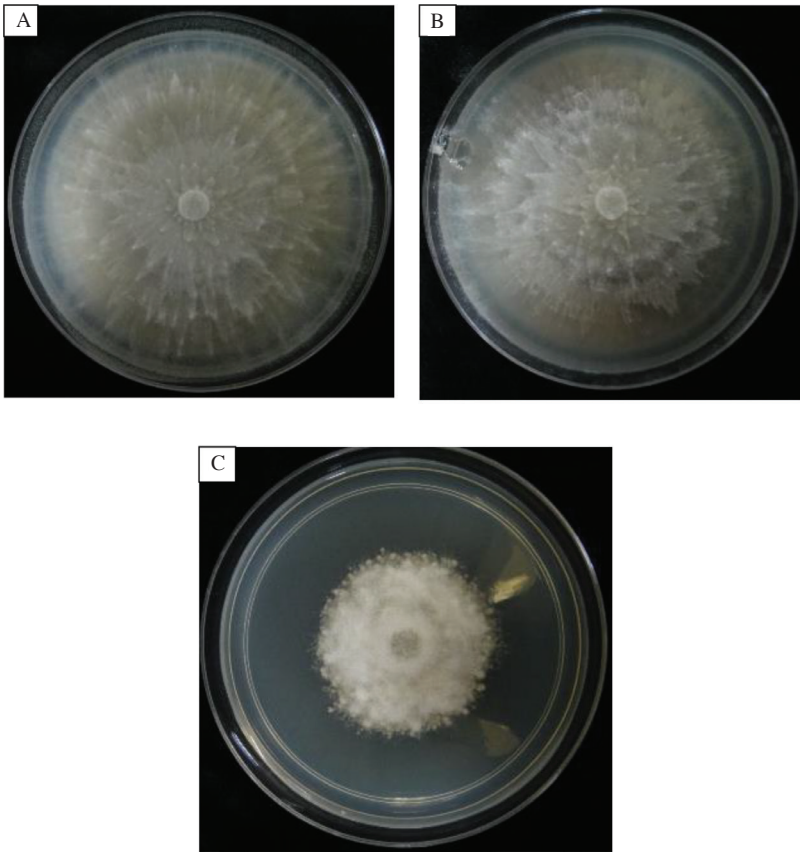

FIGURE 2. Colony morphology of *Phytophthora* spp. from cocoa (A), durian (B) and rubber (C) on PDA after seven days of incubation

TABLE 1. Means of mycelial linear growth of *Phytophthora* spp. isolates from cocoa, durian and rubber on corn meal agar at different temperatures after five days incubation

Isolate	Temperature (°C)						Host
	16	20	24	28	32	36	
DSC1	34.00±0.58e	55.67±0.44c	81.58±1.10b	89.67±0.21a	37.83±0.49d	0.00±0.00f	Durian
DSCB2	33.33±0.40d	58.67±0.40c	79.58±1.11b	89.50±0.34a	29.33±0.44e	0.00±0.00f	
DSCB4	32.42±0.30e	58.75±0.31c	80.08±0.84b	89.17±0.33a	36.17±0.37d	0.00±0.00f	
DSCE1	31.75±0.46d	55.92±0.33c	79.00±0.62b	89.25±0.31a	30.42±0.47d	0.00±0.00e	
DSCE4	34.26±0.31d	55.08±0.37c	78.67±0.79b	89.42±0.49a	29.08±0.20e	0.00±0.00f	
DSC010	34.83±0.33d	58.08±0.49c	80.08±1.31b	89.17±0.54a	36.17±0.59d	0.00±0.00e	
CPR25	21.58±0.45d	45.00±0.29c	71.92±0.72b	86.42±0.47a	21.08±0.42d	0.00±0.00e	Cocoa
CPR244	24.08±0.33d	45.50±0.43c	71.92±0.49b	88.67±0.44a	18.58±0.51d	0.00±0.00e	
CPR22	24.75±0.38d	45.83±0.33c	73.08±1.11b	88.42±0.45a	19.67±0.53e	0.00±0.00f	
CPR1003	22.67±0.73d	47.42±0.30c	71.00±0.73b	85.67±0.40a	16.00±0.29e	0.00±0.00f	
CPR140	24.83±0.33d	46.00±0.29c	72.08±0.70b	88.43±0.38a	21.42±0.51e	0.00±0.00f	
CPR211	21.58±0.40d	46.33±0.44c	71.42±0.54b	88.17±0.38a	19.08±0.49e	0.00±0.00f	
R1A	47.08±0.37e	60.42±0.42d	83.00±1.00b	90.00±0.00a	70.17±0.63c	49.00±0.93e	Rubber
R1B	48.33±0.44e	60.50±0.64d	83.58±0.37b	90.00±0.00a	69.75±0.44c	49.92±0.88e	
R2A	45.42±0.37f	60.92±0.54d	81.92±0.77b	90.00±0.00a	67.83±0.56c	51.25±0.67e	
R3A	46.83±0.46e	61.50±0.56d	81.33±0.77b	90.00±0.00a	69.50±0.76c	49.29±1.20e	
R3B	46.58±0.49f	62.08±0.61d	83.08±0.73b	90.00±0.00a	69.33±0.56c	50.00±0.65e	
R4A	48.08±0.33e	60.83±0.56[d]	82.42±0.57b	90.00±0.00[a]	69.08±0.30c	48.88±0.79e	

*Means with same alphabet among growth linear are not significantly different at $p \leq 0.05$ based on Tukey test.
**Average of six replicates of plates

media (Supplementary Data 4), which distinguished them from other *Phytophthora* species recorded in Malaysia such as *P. meadii*, *P. hevea* and *P. botryosa* which do not or rarely produce chlamydospores in media (Waterhouse 1974).

Phytophthora isolates from cocoa, rubber and durian varied slightly in size and shape of its sporangia. The sporangium from cocoa isolates were mostly ovoid (Figure 3(B)) with a length–to–breadth (L/B) ratio ranging at 1.51-1.61 with a mean 1.56 (Supplementary Data 1). Sporangia isolated from durian were larger with an L/B ratio ranging at 1.54-1.69 with a mean 1.62 (Supplementary Data 2). Most of the sporangia were ellipsoid shape (Figure 3(A)). Sporangia from rubber isolates were smaller with the mean L/B ratio at 1.34 ranging at 1.30-1.38 (Supplementary Data 3) and were sub-supherical in shape (Figure 3(C)).

All isolates from cocoa and durian possessed caducous sporangia, readily detaching itself from the sporangiophore with short hyline pedicels (Figure 3(A) & 3(B)). Mean pedicel length of isolates from cocoa and durian were 2.61±0.04 and 2.67±0.06 µm, respectively (Supplementary Data 1, 2). However, isolates from rubber exhibited non-caducous sporangia (Figure 3(C)) and no pedicel was observed. Furthermore, durian and cocoa isolates featured caducous sporangia with short pedicels <3 µm and were able to form stellate and striate colonies similar to the traits described in the Morphological Form 1 (MF1) for the *P. palmivora* group (Brasier & Griffin 1979; Newhook 1978; Waterhouse et al. 1983).

MOLECULAR IDENTIFICATION

Amplifying the ITS region using universal primers ITS6 and ITS4 produced DNA fragments 800-900 bp in size (Figures 4 & 5). These results were in agreement with Bowman et al. (2007) and Cooke et al. (2000) whom reported that amplification with ITS6 and ITS4 primer pair yielded a PCR product from *P. palmivora* and *P. nicotianae* of approximately 900 bp.

Two different species of *Phytophthora* known as *P. palmivora* and *P. nicotianae* were identified using the ITS sequences. A NJ tree based on the ITS sequences illustrated that *P. palmivora* and *P. nicotianae* were clearly from two

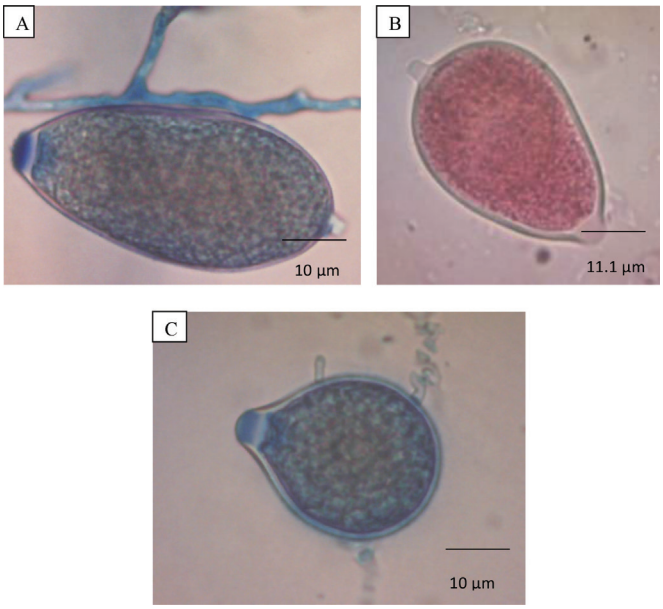

FIGURE 3. Sporangium of *Phytophthora* isolate from cocoa (A) and durian (B) was caducous with prominent papilla and short pedicel and from (C) rubber isolates was noncoducous with prominent papilla

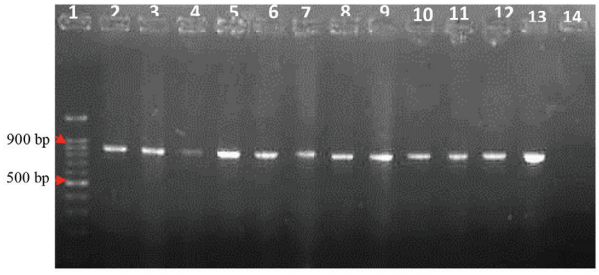

FIGURE 4. PCR products amplified with ITS6/ITS4 primers showing band approximately 850–900 bp on 1.5% agarose gel. Lane 1 = 100 bp DNA ladder, lanes 2–7 = *Phytophthora palmivora* isolates from cocoa, lanes 8–3 = *Phytophthora palmivora* isolates from durian and lane 14 = non-template control

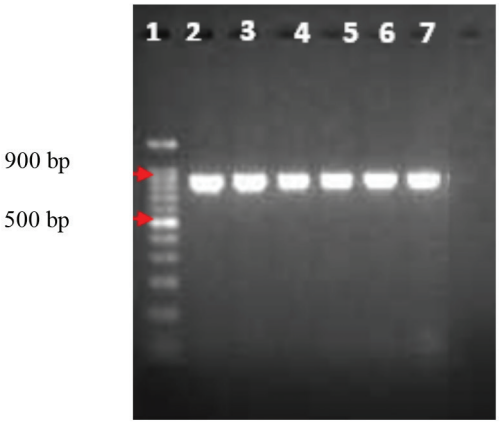

FIGURE 5. PCR products amplified with ITS6/ITS4 primers showing band approximately 900 bp on 1.5% agarose gel. Lane 1 = 100bp DNA ladder and lanes 2–7 = *Phytophthora nicotianae* isolates from rubber

distinct clades with high bootstrap value 100% (Figure 6). Both *P. palmivora* and *P. nicotianae* have been previously classified under group II by Waterhouse (1963) based on the sporangium, antheridium and reproductive behavior. However, *P. palmivora* (Clade A) and *P. nicotianae* (Clade B) were separated into different clades in the phylogenetic tree based on ITS sequences (Cooke et al. 2000; Crawford et al. 1996; Förster et al. 2000; Lee & Taylor 1992). The finding from this study is similar to Bowman et al. (2007) which successfully differentiated both *P. palmivora* and *P. nicotianae* using restriction fragment length polymorphism (RFLP) on the ITS region for species identification.

PATHOGENICITY TESTS

Pathogenicity tests for 18 isolates on detached durian and rubber leaves and unripe cocoa pods showed progressive lesion development on original host. Lesions developed in all wounded detached leaves and unripe pods regardless of the host and isolates in the cross-pathogenicity test. However, difference in the length of lesion development was observed between hosts and potential hosts. The lesion was longer in the host compared to the potential host. Summarized data of lesion length on detached cocoa pods and durian and rubber leaves are presented in Tables 2, 3 and 4. Disease severity exhibited that, on the host, the *Phytophthora* isolates were more virulent compared to the potential host (Tables 5, 6, 7). There were no lesions observed for uninoculated agar plugs (control) on detached cocoa pods, and durian and rubber leaves. The pathogenicity of *Phytophthora* spp. was confirmed via

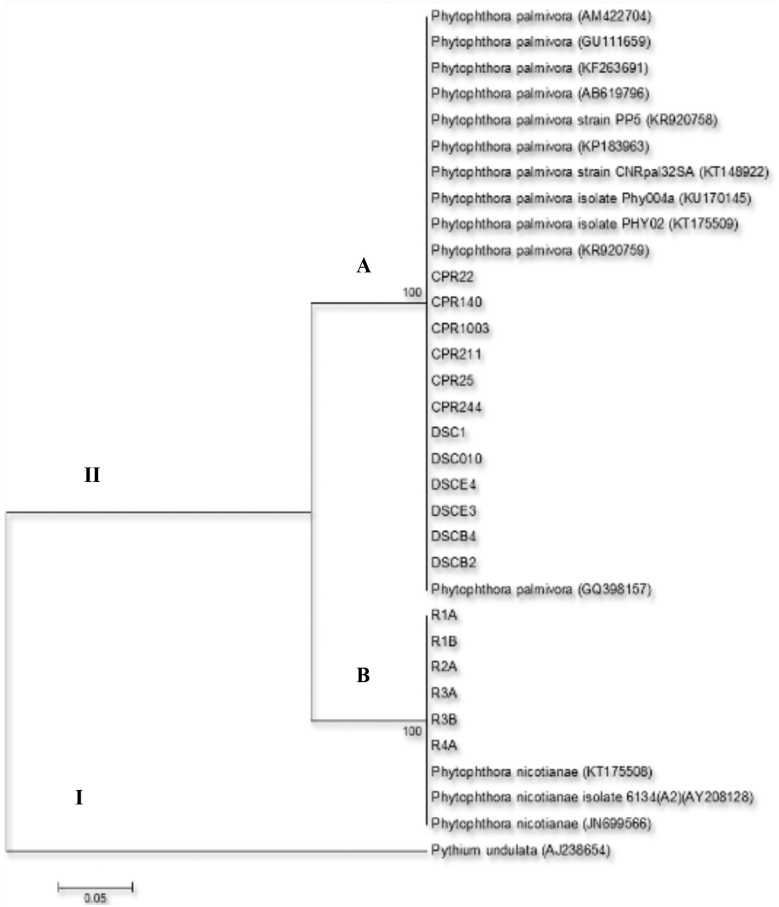

FIGURE 6. Phylogenetic tree based on ITS sequences showing the relationship among 18 *Phytophthora* isolates from cocoa, durian and rubber

TABLE 2. Mean lesion length developed on unripe cocoa pod inoculated with *Phytophthora* spp. after five days of incubation at 25±1.5°C

Isolate	Mean of lesion progress (mm/day)	*Phytophthora* spp.	Host
CPR25	18.60±0.47a	*P. palmivora*	Cocoa
CPR244	18.48±0.45a	*P. palmivora*	Cocoa
CPR140	18.32±0.73a	*P. palmivora*	Cocoa
CPR22	17.52±0.77a	*P. palmivora*	Cocoa
CPR211	17.36±0.68a	*P. palmivora*	Cocoa
CPR1003	16.40±1.03a	*P. palmivora*	Cocoa
DSC1	11.60±0.75b	*P. palmivora*	Durian
DSCE4	10.92±0.44bc	*P. palmivora*	Durian
DSCE3	10.44±0.33bc	*P. palmivora*	Durian
DSCB2	10.24±0.12bc	*P. palmivora*	Durian
DSCB4	9.92±0.27bc	*P. palmivora*	Durian
R4A	9.60±0.64bcd	*P. nicotianae*	Rubber
DSC010	9.52±0.40bcde	*P. palmivora*	Durian
R3B	8.48±0.80cdef	*P. nicotianae*	Rubber
R2A	6.92±0.34def	*P. nicotianae*	Rubber
R3A	6.56±0.17ef	*P. nicotianae*	Rubber
R1B	6.48±0.69f	*P. nicotianae*	Rubber
R1A	6.40±0.62f	*P. nicotianae*	Rubber
Control	0.00±0.00g		

Means within columns with the same letters are not significantly different by Tukey's Studentized Range (HSD) Test at $p \leq 0.05$. Mean of lesions were calculated with five independent replicates of cocoa pods

TABLE 3. Mean lesion length on rubber leaves inoculated with *Phytophthora* spp. after three days incubation at 25±1.5°C

Isolate	Leaf lesion progress (mm / day)	*Phytophthora* spp.	Host
R1B	5.43±0.23a	*P. nicotianae*	Rubber
R1A	5.19±0.07a	*P. nicotianae*	Rubber
R3B	5.15±0.26a	*P. nicotianae*	Rubber
R3A	5.14±0.20a	*P. nicotianae*	Rubber
R4A	5.11±0.24a	*P. nicotianae*	Rubber
R2A	4.88± 0.13ab	*P. nicotianae*	Rubber
CPR22	3.74±0.48bc	*P. palmivora*	Cocoa
CPR1003	3.69±0.29bc	*P. palmivora*	Cocoa
CPR140	3.59±0.22bcd	*P. palmivora*	Cocoa
CPR25	3.34±0.17cde	*P. palmivora*	Cocoa
CPR244	3.20±0.27cde	*P. palmivora*	Cocoa
CPR211	3.32±0.20cde	*P. palmivora*	Cocoa
DSCE3	2.55±0.31cdef	*P.palmivora*	Durian
DSCB2	2.51±0.27cdef	*P. palmivora*	Durian
DSCB4	2.46±0.29cdef	*P. palmivora*	Durian
DSCE4	2.34±0.27def	*P. palmivora*	Durian
DSC010	2.25±0.32ef	*P. palmivora*	Durian
DSC1	1.91±0.12f	*P. palmivora*	Durian
Control	0.00±0.00g		

Means within columns with the same letters are not significantly different by Tukey's Studentized Range (HSD) Test at $p \leq 0.05$. Mean of lesions were calculated with five independent replicates of rubber leaves

TABLE 4. Mean lesion length on durian leaves inoculated with *Phytophthora* spp. after three days incubation at 25±1.5°C

Isolates	Leaf lesion progress (mm/day)±M.S.E	*Phytophthora* spp.	Host
DSCB4	13.13±1.29a	*P. palmivora*	Durian
DSC010	13.07±0.73a	*P. palmivora*	Durian
DSC1	13.00±0.75a	*P. palmivora*	Durian
DSCB2	12.67±1.18a	P. palmivora	Durian
DSCE4	12.57±1.13a	*P. palmivora*	Durian
DSCE3	12.53±1.42a	*P. palmivora*	Durian
R4A	11.73±1.04ab	*P. nicotianae*	Rubber
R1B	10.80±1.15ab	*P. nicotianae*	Rubber
R2A	10.33±1.03ab	P. nicotianae	Rubber
R1A	9.07±0.29ab	*P. nicotianae*	Rubber
R3A	8.07±1.26bc	*P. nicotianae*	Rubber
R3B	7.87±0.40bc	*P. nicotianae*	Rubber
CPR25	5.22±0.57dc	*P. palmivora*	Cocoa
CPR244	4.88±0.34d	P. palmivora	Cocoa
CPR22	4.69±0.45d	*P. palmivora*	Cocoa
CPR1003	4.14±0.26d	*P. palmivora*	Cocoa
CPR211	4.00±0.24d	*P. palmivora*	Cocoa
CPR140	3.77±0.30d	*P. palmivora*	Cocoa
Control	0.00±0.00e		

Means within columns with the same letters are not significantly different by Tukey's Studentized Range (HSD) Test at $p \leq 0.05$. Means of lesions were calculated with five independent replicates of durian leaves

TABLE 5. Disease severity scored based on the developed severity scale (Figure 7) on cocoa pod inoculated with *Phytophthora* isolates isolated from cocoa, durian and rubber *in-vitro*

Isolate	Disease severity (%) on cocoa pod			
	Scale 1	Scale 2	Scale 3	Scale 4
Phytophthora isolates from cocoa	_	_	_	100%
Phytophthora isolates from durian	_	43%	10%	_
Phytophthora isolates from rubber	_	100%	_	_
Control	4%	_	_	_

TABLE 6. Disease severity scored based on the developed severity scale on rubber leaf inoculated with *Phytophthora* isolates isolated from cocoa, durian and rubber *in-vitro*

Isolate	Disease severity (%) on cocoa pod			
	Scale 1	Scale 2	Scale 3	Scale 4
Phytophthora isolates from rubber	_	_	23%	70%
Phytophthora isolates from cocoa	_	8%	60%	3%
Phytophthora isolates from durian	_	38%	18%	_
Control	4%	_	_	_

TABLE 7. Disease severity scored based on the developed severity scale on durian leaf inoculated with *Phytophthora* isolates isolated from cocoa, durian and rubber *in-vitro*

Isolate	Disease severity (%) on cocoa pod			
	Scale 1	Scale 2	Scale 3	Scale 4
Phytophthora isolates from durian	_	_	5%	93%
Phytophthora isolates from cocoa	_	40%	15%	_
Phytophthora isolates from rubber	_	1.7%	50%	30%
Control	4%	_	_	_

successful re-isolation from the infected tissues of detached cocoa pods and leaves.

All the *P. palmivora* isolates from durian and cocoa successfully infected its own host and potential hosts in an *in-vitro* pathogenicity test. However, *P. palmivora* isolates did not exhibit a remarkable degree of host specificity. Nonetheless, variable size of lesions was developed when these isolates inoculated on its own host and potential hosts. *P. palmivora* exhibited a longer length of lesion on its own host and conversely displayed a shorter length of lesions on potential host plants. In addition, lesion development progress on potential hosts was slower compared to its original host. Similar findings were observed by Slamet (1991) where artificial cross-inoculation of three *P. palmivora* isolates from coconut, black pepper and cocoa showed differences in virulence among the three isolates tested on the plants. A higher degree of aggressiveness was noted on the original host compared to the potential hosts. *P. palmivora* isolate from cocoa managed to infect pepper leaves with a very slow infection progress rate (Slamet 1991).

Cross-pathogenicity tests by many researchers showed that *P. palmivora* isolates causing black stripe and patch canker of rubber and leaf and collar rot of black pepper in Southeast Asia can also cause patch canker of durian (Belgrave & Norris 1917; Navaratnam 1966; Suzuki et al. 1979; Tsao & Tummakate 1977). Boccas (1973) crossed *P. palmivora* isolates and demonstrated that there were pathogenic variabilities on roselle, tomato, eggplant and melon. These previous studies suggested that it was possible for *P. palmivora* to cross-infect potential host plants with varying degrees of virulence in pathogenicity. According to McMahon and Purwantara (2004), *P. palmivora* population may contain a range of strains and only a few are pathogenic and able to infect more than one host, while others are host-specific.

The isolated *P. nicotianae* strains from rubber in our study exhibited cross-inoculation infectivity on cocoa pods and durian leaves. However, lesion formation progress was slow compared to its host. Hall (1993) noted that *P. nicotianae* is a destructive pathogen of a wide range of predominantly dicotyledonous crops, ornamental plants and herbaceous and woody members in many plant families. *P. nicotianae* causes patch canker in durian and fruit rot, crown rot, foot rot, root rot in black pepper especially in Malaysia and Thailand (Liu 1977; Suzuki et

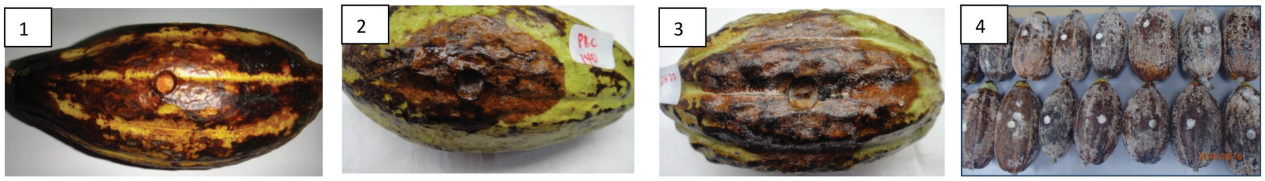

FIGURE 7. Scale for disease severity on cocoa pod caused by *Phytophthora* sp. (1) no visible cocoa lesion, (2) initial infection of cocoa pod rot with lesion < 12 mm in length, (3) lesion disperse around cocoa pod with lesion 12-15 mm in length, and (4) lesion progressed to entire pod with lesion >15 mm developed on cocoa pod

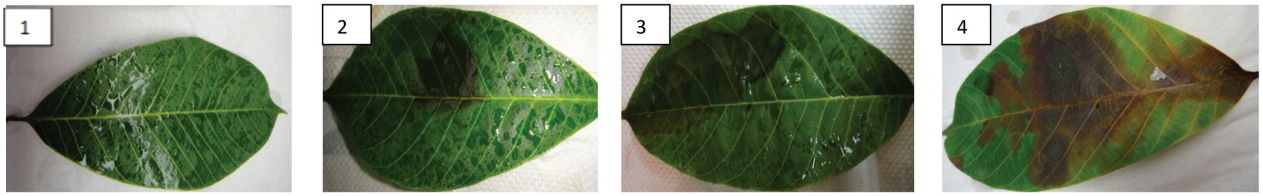

FIGURE 8. Scale for disease severity leaf on rubber leaves caused by *Phytophthora* sp. (1) healthy leaf, (2) initial lesion developed on rubber leaf with lesion <3 mm in length, (3) lesion dispersed around leaf with lesion 3-5 mm in length and (4) lesion progressed to entire leaf with discoloration with leaf lesion >5 mm in length

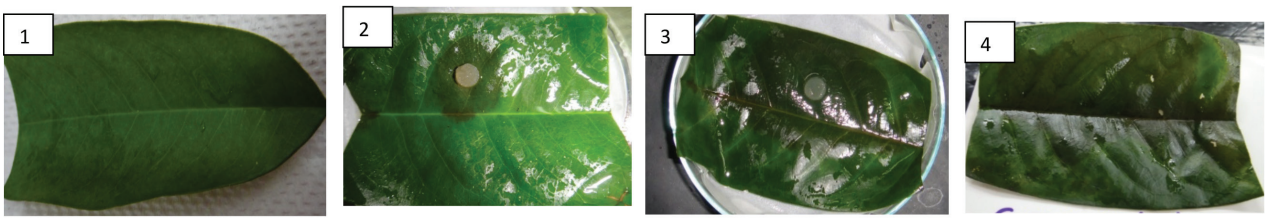

FIGURE 9. Scale for disease severity on durian leaves caused by *Phytophthora* sp. (1) healthy leaf, (2) initial infection exhibiting leaf lesion with lesion <5 mm in length, (3) lesion dispersed around leaf with lesion of 5–10 mm in length, and (4) lesion progressed to entire leaf with discoloration with lesion >10 mm in length

al. 1979). Moreover, *P. nicotianae* also causes leaf fall of rubber in India (Edathil & George 1976) and cocoa pod rot (Liu 1977). In addition, *P. nicotianae* var. *nicotianae* isolated from leaves and shoots of cashew trees caused shoot rot and leaf fall during the southwest monsoon (June-August) in South India (Thankamma 1974). Thankamma (1983) reported that *P. nicotianae* var. *nicoteanae* isolated from pomegranate, black pepper and Chinese hibiscus as well as *P. meadii* from brinjal (aubergine) and wild jack plant were pathogenic to rubber.

In this study, 18 *Phytophthora* isolates from durian stem canker, cocoa and rubber pod rot were successfully identified and characterized. In *in vitro* pathogenicity experiments from our study demonstrated that *Phytophthora* isolates were pathogenic to both host and potential host plant tissues with lesion development on the tested plant tissues. However, the lesion was more severe on host tissues compared to the potential host plants. The ability of *Phytophthora* spp. to infect potential host plants under natural conditions has not been studied. Therefore, further studies are required to confirm whether these isolated *Phytophthora* spp. were able to cause cross-pathogenicity towards potential host plants under field conditions.

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REFERENCES

Belgrave, W.N.C. & Norris, F.D.L.M. 1917. Notes on bark cankers and their treatment. *F.M.S. Agric. Bull.* 6: 2-10.

Boccas, B.R. 1973. Observations preliminaires sur l'heredité du pouvoir pathogène chez le *P. palmivora* (Butl.) Butl. Cah. *ORSTOM sér. Bio.* 20: 51-56.

Bowman, K.D., Albrecht, U., Graham, J.H. & Bright, D.B. 2007. Detection of *Phytophthora nicotianae* and *P. palmivora* in citrus roots using PCR-RFLP in comparison with other methods. *Eur. J. Plant Pathol.* 119: 143-158.

Brasier, C.M. & Griffin, M.J. 1979. Taxonomy of '*Pytophthora palmivora*' on cocoa. *Trans. Br. Mycol. Soc.* 72: 111-143.

Chee, K.H. 1973. Production, germination and survival of chlamydospores of *Phytophthora palmivora* from *Hevea brasiliensis*. *Trans. Br. Mycol. Soc.* 6: 21-26.

Chillali, M., Idder-Ighili, H., Guillaumin, J.J., Mohammed, C., Escarmant, L. & Botton, B. 1998. Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European *Armillaria*. *Mycol. Res.* 102(5): 533-540.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. & Brasier, C.M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genet. Bio.* 30: 17-32.

Crawford, A.R., Bassam, B.J., Drenth, A., MacLean, D.J. & Irwin, J.A.G. 1996. Evolutionary relationships among *Phytophthora* species deduced from rDNA sequence analysis. *Mycol. Res.* 100: 437-443.

Drenth, A. & Sendall, B. 2004. Economic impact of *Phytophthora* diseases in Southeast Asia. In *Diversity and Management of Phytophthora in Southeast Asia*, edited by Drenth, A. & Guest, D.I. *ACIAR Monograph*. 114: 10-28, 97-99.

Edathil, T.T. & George, M.K. 1976: *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse on *Hevea brasiliensis* in South India. Rubb. *Board Bull.* 13: 3-4.

Erwin, D.C. & Ribeiro, O.K. 1996. *Phytophthora Diseases Worldwide*. St. Paul, Minnesota: The American Phytopathological Society.

Förster, H., Cummings, M.P. & Coffey, M.D. 2000. Phylogenetic relationship of *Phytophthora* species based on ribosomal ITS I DNA sequence analysis with emphasis on Waterhouse groups V and VI. *Mycol. Res.* 104: 1055-1061.

Hall, G.1993. An integrated approach to the analysis of variation in *Phytophthora nicotianae* and a redescription of the species. *Mycol. Res.* 97: 559-574.

Henson, J.M. & French, R. 1993. The polymerase chain reaction and plant disease diagnosis. *Annu. Rev. Phytopathol.* 31: 81-109.

Hee, W.Y., Torreña, P.S., Blackman, L.M. & Hardham, A.R. 2013. *Phytophthora cinnamomi* in Australia. In *Phytophthora: A Global Perspective,* edited by Lamour, K. UK: CAB International. pp. 124.

Hibbett, D.S. 1992. Ribosomal RNA and fungal systematics. *Trans. Mycol. Soc. Japan* 33: 533-556.

Ko, W.S. & Ho, W.C. 1997. A simple method for obtaining single-spores isolates of fungi. *Bot. Bull. Acad. Sinica.* 38: 41-44.

Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. 1990. *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, Inc.

Jiang, R.H., Tripathy, S., Govers, F. & Tyler, B.M. 2008. RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving superfamily with more than 700 members. *Proceedings of the National Academy of Sciences of the United States of America* 105: 4874-4879.

Kennedy, D.M. & Duncan, J.M. 1995. A papillate *Phytophthora* species with specificity to Rubus. *Mycol. Res.* 99: 57-68.

Lee, B.S. & Varghese, G. 1974. Studies on the genus *Phytophthora* in Malaysia. II. Reproduction and sexuality. *Malay. Agric. Res.* 3: 137-149.

Lee, S.B. & Taylor, J.W. 1992. Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. *Mol. Biol. Evol.* 9: 636-653.

Liu, P.S.W. 1977. Diseases caused by *Phytophthora* and *Pythium* in Sabah, Malaysia. *Tech. Bull.* 3: 48.

MacDonald, J.D., Stites, J. & Kabashima, J. 1990. Comparison of serological and culture plate methods for detection species of *Phytophthora*, *Pythium*, and *Rhizoctonia* in ornamental plants. *Plant Dis.* 74: 655-659.

McMahon, P. & Purwantara, A. 2004. *Phytophthora* on cocoa. In *Diversity and Management of Phytophthora in Southeast Asia*, edited by Drenth, A. & Guest, D.I. *ACIAR Monograph* 114: 104-115.

Navaratnam, S.J. 1966. Patch canker of the durian tree. *Malay. Agric. J.* 45: 291-294.

Newhook, F.J. 1978. *Phytophthora cinnamomi* in native forests of Australia and New Zealand: Indigenous or introduced? In *Microbial Ecology,* edited by Loutit, M.W. & Heidelberg, J.A.R. Germany: Springer-Verlag.

Oliver, R.P. 1993. Nucleic acid-based methods for detection and identification. In *Principles of Diagnostic Techniques*

in Plant Pathology, edited by Fox, R.T.V. Wallingford, UK: CAB International. pp. 153-170.

Raffaele, S., Farrer, R.A. & Cano, L.M. 2010. Genome evolution following host jumps in the Irish potato famine pathogen lineage. *Science* 330: 1540-1543.

Seidl, M.F., van den Ackerveken, G., Govers, F. & Snel, B. 2011. A domain-centric analysis of oomycete plant pathogen genomes reveals unique protein organization. *Plant Physiol*. 155: 628-644.

Shearer, B.L., Crane, C.E. & Cochrane, A. 2004. Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi*. *Aust. J. Bot*. 52: 435-443.

Slamet, A.R. 1991. Pathogenicity test of three isolates of *Phytophthora palmivora* on black pepper, coconut, cacao and vanilla. *Bulletin Komunikasi Penetilian Rempah dan Obat*. p. 6.

Stamps, D.J., Waterhouse, G.M., Newhook, F.J. & Hall, G.S. 1990. Revised tabular key to the species of *Phytophthora*. *Mycol. Papers* 162: 1-28.

Suzuki, T.J., Kueprakone, U. & Kamphangridthrong, T. 1979. *Phytophthora* spp. isolated from some economic plants in Thailand. *Tech. Bull. Trop. Agric. Res. Center Jpn*. 12: 32-41.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA 6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*. 30(12): 2725-2729.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D. & Fisher, M.C. 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genet. Biol*. 31: 21-32.

Thankamma, L. 1974. *Phytophthora nicotianae* var. *nicotianae* on *Anacardium occidentale* in south India. *Plant Dis. Rep*. 58: 767-768.

Thankamma, L. 1983. *Phytophthora* species on eight indigenous host species in south India and their pathogenicity on rubber. *Indian Phytopathol*. 36: 17-23.

Tsao, P.H. & Ocana, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature* 223: 636-638.

Tsao, P.H. & Tummakate, R. 1977. The identity of a *Phytophthora* species from black pepper in Thailand. *Mycologia* 69: 631-637.

Waterhouse, G.M. 1974. *Phytophthora palmivora* and some related species. In *Phytophthora Disease of Cocoa*, edited by Gregory, P.H. London: Longman. pp. 51-70.

Waterhouse, G.M. 1963. Key to the species of *Phytophthora* de Bary. *Mycol. Papers*. 92: 1-22.

Waterhouse, G.M., Newhook, F.J. & Stamps, D.J. 1983. Present criteria for classification of *Phytophthora*. In *Phytophthora*: *Its Biology, Taxonomy, Ecology, and Pathology*, edited by Erwin, D.C., Bartnicki-Garcia, S. & Tsao, P.H. St. Paul, Minnesota: The American Phytopathological Society. pp. 139-147.

Weste, G. 1983. Population dynamics and survival of *Phytophthora*. In *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology*, edited by Erwin, D.C., Bartnicki-Garcia, S. & Tsao, P.H. St. Paul, Minnesota: The American Phytopathological Society. pp. 139-147.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications,* edited by Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. San Diego: Academic Press. pp. 315-322.

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Supplementary Data 1. Sporangial morphology of *Phytophthora* spp. from cocoa isolates after seven days of incubation in the light at28±1.5°C

Isolate	Extreme range of length × breadth (µm)	*Mean length (µm) ± M.S.E	*Mean breadth (µm) ± M.S.E	l/b ratio	*Mean pedicel length (µm) ± M.S.E	*Mean apical depth (µm) ± M.S.E	*Mean width of exit Pore (µm) ± M.S.E
CPR244	35.88 – 51.96 × 26.23 – 32.17	41.584± 0.39	27.38 ± 0.13	1.52	2.55 ± 0.03	2.73 ± 0.05	4.61 ± 0.10
CPR25	39.59 – 50.72 × 27.22 – 35.88	45.45 ± 0.35	30.03 ± 0.23	1.51	2.67 ± 0.06	2.76 ± 0.07	4.59 ± 0.09
CPR22	40.83 – 49.49 × 27.96 – 33.40	45.77 ± 0.32	29.79 ± 0.19	1.54	2.71 ± 0.07	2.89 ± 0.08	4.69 ± 0.09
CPR1003	37.11 – 48.99 × 25.24 – 30.68	43.98 ± 0.41	28.40 ± 0.20	1.55	2.51 ± 0.02	2.76 ± 0.07	4.84 ± 0.06
CPR211	36.62 – 50.22 × 24.74 – 33.89	46.83 ± 0.40	29.05 ± 0.27	1.61	2.60 ± 0.05	2.77 ± 0.08	4.80 ± 0.06
CPR140	35.87 – 54.43 × 25.98 – 34.64	45.16 ± 0.40	29.64 ± 0.25	1.52	2.64 ± 0.05	2.70± 0.06	4.70 ± 0.06

*Average of 100 sporangia measurements

Supplementary Data 2. Sporangial morphology of *Phytophthora* spp. from durian isolates after seven days of incubation in the light at 28±1.5°C

Isolates	Extreme range of length × breadth (µm)	*Mean length (µm) ± M.S.E	*Mean breadth (µm) ± M.S.E	l/b ratio	*Mean pedicel length (µm) ± M.S.E	*Mean apical depth (µm) ± M.S.E	*Mean width of exit pore (µm) ± M.S.E
DSC 1	44.53 – 70.51 × 25.98 – 35.38	52.16 ± 0.63	30.84 ± 0.28	1.69	2.58 ± 0.05	2.67 ± 0.06	5.00 ± 0.04
DSC B2	51.02 – 76.53 × 33.16 – 42.09	60.33 ± 0.68	37.35 ± 0.29	1.62	2.65 ± 0.05	2.80 ± 0.04	5.26 ± 0.04
DSC B4	45.92 – 66.33 × 28.70 – 37.76	55.38 ± 0.51	33.76 ± 0.20	1.63	2.70 ± 0.06	2.65 ± 0.05	5.61 ± 0.10
DSC E3	40.82 – 79.08 × 28.06 – 45.92	59.29 ± 0.86	36.63 ± 0.48	1.63	2.72 ± 0.06	2.86 ± 0.06	4.95 ± 0.080
DSC E4	43.37 – 68.88 × 26.51 – 40.82	56.35 ± 0.65	34.99 ± 0.39	1.63	2.76 ± 0.07	2.81 ± 0.08	5.36 ± 0.08
DSC 010	44.37 – 63.78 × 27.52 – 44.82	53.16 ± 0.65	34.90 ± 0.41	1.54	2.73 ± 0.07	2.74 ± 0.07	5.26 ± 0.06

*Average of 100 sporangia measurement